Culture media and sources of nitrogen promoting the formation of stromata and ascocarps in *Petromyces alliaceus* (*Aspergillus* section *Flavi*)

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Abstract: *Petromyces alliaceus* Malloch and Cain is the only known sexually reproducing fungus classified in *Aspergillus* section *Flavi*. The goal of this research was to identify culture media and sources of nitrogen that best support the formation of stromata with ascocarps. Three cultures of *P. alliaceus* isolated from crop field soils were grown on selected agar media in Petri dishes for 7 months at 30 °C in darkness. The largest numbers of stromata were recorded for cultures grown on Czapek's agar (CZA) and a mixed cereal agar (MCA), while the percentage of stromata containing ascocarps was greatest ($P \leq 0.05$) for cultures grown on MCA (25%–28%). When *P. alliaceus* was grown on standard CZA containing 0.3% $NaNO_3$, only 5% of the stromata contained ascocarps. A greater percentage of the stromata (15%–22%) formed ascocarps when the $NaNO_3$ in CZA was replaced with an equivalent amount of available nitrogen supplied by ammonium tartrate, glutamic acid, or serine.

Key words: ascospores, homothallic, sclerotia, essential amino acids.

Résumé : *Petromyces alliaceus* Malloch et Cain est le seule champignon connu à reproduction sexuée classifié dans les *Aspergillus* section *Flavi*. Le but de cette recherche été d'identifier les milieux de culture et la source d'azote soutenant le mieux la formation de stroma avec ascocarpes. Trois cultures de *P. alliaceus* isolées de sols agricoles furent cultivées sur les milieux gélosés sélectionnés dans des boîtes de Petri pendant 7 mois à 30 °C dans l'obscurité. Le plus grand nombre de stroma fut obtenu chez des cultures cultivées sur de l'agar de Czapek (CZA) et un agar de céréales mixtes (MCA) alors que le pourcentage de stroma contenant des ascocarpes était le plus élevé ($P \leq 0{,}05$) pour des cultures cultivées sur du MCA (25 % – 28 %). Lorsque *P. alliaceus* fut cultivé sur du CZA normal contenant 0,03 % de $NaNO_3$, seulement 5 % des stroma contenaient des ascocarpes. Un pourcentage supérieur de stroma (15 % – 22 %) ont formé des ascocarpes lorsque le $NaNO_3$ dans le CZA a été remplacé par une quantité équivalente d'azote disponible fourni par du tartrate d'ammonium, de l'acide glutamique ou de la sérine.

Mots clés : ascospores, homothallique, sclérote, acide aminé essential.

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Introduction

Petromyces alliaceus Malloch and Cain (anamorph: *Aspergillus alliaceus* Thom and Church) is the only known sexually reproducing fungus classified in *Aspergillus* section *Flavi* (Peterson 1995, 2000). When grown on solid culture media, *P. alliaceus* produces numerous grey–black sclerenchymatous stromata, some of which mature slowly over several months to form nonostiolate ascocarps with asci and ascospores (Fennell and Warcup 1959; Raper and Fennell 1965). Sexual reproduction in *P. alliaceus* is homothallic. The fungus has been recently linked to the ochratoxin A contamination that is occasionally observed in California fig (*Ficus carica* L.) orchards (Doster et al. 1996; Doster and Michailides 1998; Bayman et al. 2002). While nothing is known about ascocarp or stromata formation in the disease cycle of *P. alliaceus* in orchards or crop fields, the production of meiospores (ascospores) in stromata produced from a heterokaryotic mycelium represents an important source of genetic variation among naturally occurring clonal populations (Hoffman et al. 2001).

The purpose of this study was to identify what culture media best support the formation of *P. alliaceus* stromata with ascocarps. The stimulatory or inhibitory effect of different sources of nitrogen on *Aspergillus* mycelial growth (Ruperez and Leal 1979; Payne and Hagler 1983) or sclerotium formation (Rudolph 1962; Rai et al. 1967; Agnihotri 1968; Paster and Chet 1980; McAlpin 2001) is well documented. However, there is no information on the role of nitrogenous compounds in the initiation and development of ascocarps within stromata of *P. alliaceus*.

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Materials and methods

Fungal inoculum and culture conditions

The cultures of *P. alliaceus* examined in this study were obtained from the Agricultural Research Service Culture

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Table 1. Stromata and ascocarp production by *Petromyces alliaceus* on selected culture media at 30 °C (7 months).

	NRRL 31813		NRRL 31814		NRRL 31816	
Culture medium	Mean no. of stromata	Stromata with ascocarps (%)[a]	Mean no. of stromata	Stromata with ascocarps (%)[a]	Mean no. of stromata	Stromata with ascocarps (%)[a]
Coons medium 1 (CM1)	696f	9	912d	7b	732d	8c
Coons medium 2 (CM2)	598f	4d	643e	5	553d	3d
Corn meal agar (CMA)	53g	0	25f	0	32e	0
Czapek's agar (CZA)	2672a	4d	2766a	5c	2457a	4d
Complete medium (CYM)	1605d	4d	1594c	5c	1549c	4d
Malt extract agar (MEA)	657f	1	745d	1d	812d	0
Mixed cereal agar (MCA)	2481b	25a	2640a	26a	2341a	28a
Oatmeal agar (OA)	2239c	11b	2294b	8b	2172a	12b
Potato dextrose agar (PDA)	2218c	3d	1712c	3d	1866b	3d
PDA + yeast extract (PDAYE)	822e	3d	784d	2d	664d	2d
Sabouraud's agar (SA)	136g	0	118f	0	151e	0
V8 juice agar (V8)	102g	0	72f	0	66e	0
***F* value**	281.8	167.3	214.3	154.7	237.5	106.2

Note: Means within a column followed by the same letter are not significantly different based on Tukey's multiple comparison test ($P = 0.05$). All values are the mean of four replicate plates.

[a]Percentage of stromata having at least one ascocarp.

Collection (NRRL). The cultures were isolated in 1996 from crop field soils and first shown to produce stromata with ascocarps by Dr. B.W. Horn, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia. These include NRRL 31813 and NRRL 31814 isolated from cotton field soil samples collected in eastern New Mexico and NRRL 31816 isolated from peanut field soil collected near Dawson, Georgia. In selecting these isolates, we sought to avoid cultural degeneration resulting from periodic transfer. The cultures were maintained on silica gel prior to being received by the ARS Culture Collection, 24 January 2002. Each isolate produces a unique DNA fingerprint (pAF28 DNA probe) and represents a distinct genotype or clonal population (McAlpin and Wicklow 2005). Conidia from 5-day-old cultures of each strain grown on potato dextrose agar (PDA) slants incubated at 30 °C were harvested after adding 5 mL of 0.1% water agar to each slant. Cultures were then agitated for 20 s using a vortex and the spore suspensions transferred to sterile screw-capped culture tubes and adjusted to 1×10^5 spores/mL with sterile 0.1% water agar.

The following media were used to test the influence of a variety of natural materials on the production of stromata with ascocarps by *P. alliaceus*: Coon's medium for *Fusarium* (CM) (Booth 1971), a modification of Coon's medium (CMM) with 7.2 g of maltose and 10 g of potato starch replacing saccharose and dextrose as sources of carbon, corn meal agar (CMA) (Difco), Czapek's agar (CZA) (Difco), complete medium (CYM) (CZA plus 0.25% yeast extract plus 0.75% maltose) (Papa 1986), malt extract agar (MEA) (Atlas 1993), mixed cereal agar (MCA) (50 g of Gerber mixed grain cereal (Gerber Products Co., Freemont, Michigan) plus 15 g agar/L), oatmeal agar (OA), 60 g of ground oatmeal, 12 g agar/L (= ATCC medium 551), PDA (Difco), PDA plus 1% yeast extract (PDAYE), modified from Gomez-Miranda and Leal (1981), Sabouraud's agar (SA) (Difco), and V8 juice agar (V8) (Atlas 1993). All culture media used in these experiments were adjusted to pH 7.0 using 5 mol NaOH/L or 5 mol HCl/L. Preliminary studies revealed the optimal production of stromata with ascocarps on CZA adjusted to pH 7.0 with dark incubation at 30 °C. Twenty-five millilitres of culture medium was added to each of 12 Petri dishes (9 cm in diameter) providing four replicate cultures for each of the three isolates of *P. alliaceus*. Petri dishes were centrally inoculated with 5 µL of an appropriate spore suspension and incubated in plastic trays covered with aluminum foil at 30 °C in the dark.

Examination of stromata

All stromata had formed after 3 weeks of incubation and were counted with the aid of a dissecting microscope and counting grid. With the great majority of cultures, the stromata were distributed uniformly throughout the colony. This enabled us to count the stromata in sections of each colony (halves or quarters) and apply appropriate multiples to approximate the total. When stromata were unevenly distributed within a colony, or relatively few stromata were produced, all of the stromata were counted. Cultures were examined for stromata with ascocarps at 8, 12, 16, 20, 24, and 28 weeks following inoculation. At least 20 of the stromata produced by each isolate of *P. alliaceus* on the different culture media were carefully removed using fine-tipped forceps and examined for evidence of stromata ripening or the formation of ascocarps. Softening of the sclerenchymatous stromatal tissues after week 12 was indicative of near ascocarp maturity. The release of a creamy exudate from individual stromata that were crushed with forceps after week 16 was evidence that mature ascocarps had formed. This was confirmed microscopically by the presence of asci and ascospores. After week 20, we randomly selected and individually crushed at least 100 stromata from each replicate culture of the different culture media (Table 1). Stromata were first washed in 0.01% Triton X-100 to remove the *Aspergillus* conidia and then air dried in a laminar flow safety cabinet prior to being examined for the presence of asci and ascospores. For some of the media that supported fewer than 300 stromata per culture dish (i.e., CMA, CZA–cysteine,

Table 2. Stromata and ascocarp production by *Petromyces alliaceus* on nitrogen-amended Czapek's agar at 30 °C (7 months).

	NRRL 31813		NRRL 31814		NRRL 31816	
Nitrogen source	Mean no. of stromata	Stromata with ascocarps (%)[a]	Mean no. of stromata	Stromata with ascocarps (%)[a]	Mean no. of stromata	Stromata with ascocarps (%)[a]
Alanine	1425cd	5c	1505d	8c	2147c	6d
Arginine	1801c	10b	675e	10b	1569d	12c
Asparagine	2313b	5c	1607d	13b	2721b	6d
Aspartic acid	988de	5c	1554d	7c	1200d	11c
Cysteine	13f	0	27e	0	24e	0
Cystine	0	0	0	0	0	0
Glutamic acid	1129d	17a	1193d	22a	1048d	16b
Glutamine	1130d	7b	2322c	7c	2389bc	6d
Glycine	2729a	13b	2661c	20a	2418b	16b
Histidine	2578ab	9b	1105d	10b	2193c	11c
Hydroxyproline	1588c	11b	3923a	9bc	2775b	10c
Isoleucine	2414b	5c	1612d	6cd	2500b	4e
Leucine	2860a	5c	3605b	6cd	4684a	3e
Lysine	297e	14ab	278e	11b	248e	4e
Methionine	1215cd	4c	1380d	7c	2579b	4e
Phenylalanine	2334b	4c	2688c	4d	2695b	4e
Proline	2499b	7c	1544d	8c	2294bc	5de
Serine	1949c	17a	1262d	20a	2916bc	15b
Threonine	2439b	3c	3215c	6cd	2218c	8cd
Tryptophan	1320cd	5c	1490d	5d	2393bc	6d
Tyrosine	2287b	5c	3200c	5d	2479b	5de
Valine	1774c	5c	1183d	4d	1033d	5e
Ammonium nitrate	509e	7bc	260e	7c	551e	5e
Ammonium sulphate	0	0	0	0	0	0
Ammonium tartrate	1393cd	18a	1290d	19ab	1093d	22a
Hypoxanthine	2361b	10b	2945c	12b	2748b	10c
Potassium nitrate	2941a	6c	2409c	5d	3402b	6d
Sodium nitrate	2600ab	6c	3148c	5d	2337c	5de
Urea	1019d	4c	1013d	4d	780d	3e
Control (no nitrogen)	3f	0	4e	0	3e	0
***F* value**	94.07	28.56	117.10	39.96	48.68	30.25

Note: Means within a column followed by the same letter are not significantly different based on Tukey's multiple comparison test ($P = 0.05$). All values are the mean of four replicate plates.

[a]Percentage of stromata having at least one ascocarp.

CZA–lysine, CZA – no nitrogen, and SA), sterile forceps were used in applying pressure directly to about 20 stromata within each culture dish for evidence of ripening. Using this nondestructive approach, we could repeatedly test some of the same stromata at earlier incubation intervals before removing and crushing equivalent numbers of these stromata after weeks 20 and 24.

An evaluation of sources of nitrogen promoting stromata and ascocarp formation in *P. alliaceus* was performed using CZA as the basal medium. The $NaNO_3$ in CZA was replaced with one of 22 amino acids or six nitrogen-containing compounds in amounts calculated to provide an equivalent amount of available nitrogen (CZA = 0.49 g N/L) and then adjusted to pH 7.0 before autoclaving (Table 2). The basal medium CZA served as the basis for evaluating other sources of nitrogen, while CZA having no nitrogen served as a control. Replicate cultures of *P. alliaceus* isolates were prepared for media containing each source of nitrogen and controls as described above.

Statistical analyses

Numbers of stromata and the percentage of stromata containing ascocarps on different culture media and sources of nitrogen were compared by ANOVA at the $P = 0.05$ level. Where a significant ($P < 0.01$) F value was found in the ANOVA, this was followed by a post-test using Tukey's multiple comparison test (Graph Pad Software Inc., San Diego, California). The least significant difference (LSD) was also calculated for each isolate to verify differences in the means. Means that were not significantly different were assigned the same letter.

Results

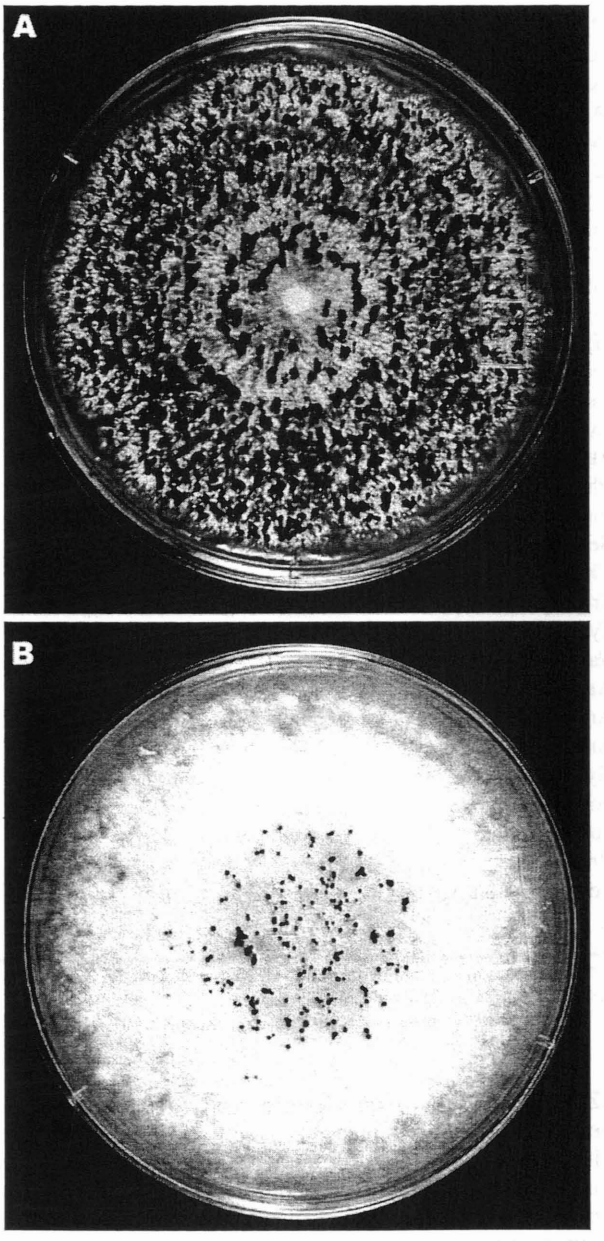
Three cultures of *P. alliaceus* (NRRL 31813, NRRL 31814, and NRRL 31816) were grown on selected culture media containing a variety of natural materials in Petri dishes including CZA amended with different sources of nitrogen (7 months of dark incubation, 30 °C). Stromata initials

developed after 5 days forming numerous globose to subglobose or sometimes elongated soft stromata. The stromata were mostly hardened after 14 days and begun to turn grey–black in color. All of the stromata on each culture medium completed the initial "sclerotial" phase of maturation at 21 days. The largest numbers of stromata per Petri dish were recorded for CZA (mean numbers = 2457–2766) (Fig. 1) and MCA (mean numbers = 2341–2640) (Table 1). The maturation of stromata to form ascocarps progressed slowly on each of the media that supported the formation of stromata with ascocarps (Table 1). The hardened stromata could not be crushed during the first 8 weeks of incubation. After 14 weeks, some of the stromata were found to have softened and microscopic observations revealed the presence of ascocarps with immature asci. The numbers of stromata containing mature ascocarps with ascospores were recorded for all fungal isolates and culture media during weeks 16–22, with the peak occurring at 20 weeks. No further increases in the percentage of stromata containing ascocarps were recorded at 24 or 28 weeks, after which no further observations were performed. No evidence was found that any of the culture media supported a faster rate of ascocarp ripening. The percentage of stromata containing ascocarps was greatest ($P \leq 0.05$) for MCA (25%–28%) (Table 1). Large numbers of stromata were also recorded for cultures grown on OA (mean numbers = 2172–2294) and PDA (mean numbers = 1712–2218). The percentage of stromata containing ascocarps was significantly greater ($P \leq 0.05$) for cultures grown on OA (8–12%) than those grown on PDA (3%). The fewest stromata, none of which contained ascocarps, were recorded for cultures grown on CMA (mean numbers = 25–53), V8 (mean numbers = 66–102), and SA (mean numbers = 118–151).

When the *P. alliaceus* cultures were grown on CZA in which $NaNO_3$ was replaced with different sources of nitrogen, the greatest ($P \leq 0.05$) numbers of stromata produced by NRRL 31813 were on glycine (mean = 2729), leucine (mean = 2860), histidine (mean = 2578), and potassium nitrate (mean = 2941), NRRL 31814 on hydroxyproline (mean = 3923), and NRRL 31816 on leucine (mean = 4684) (Table 2). Stromata produced with leucine as the source of nitrogen were consistently smaller in size (0.5 mm) when contrasted with the majority of stromata produced on the other media (0.5–4.0 mm × 0.5–1.0 mm). The stromata produced with potassium nitrate commonly occurred in clusters and proved more difficult to count.

When cultured on standard CZA containing 0.3% $NaNO_3$, 5% of the stromata that had formed after 21 days were found to contain from one to several ascocarps with continued incubation for up to 5 months (Table 2). Nitrogen sources supporting the fewest stromata per culture plate included cysteine (mean numbers = 13–27), lysine (mean numbers = 248–297) (Fig. 1), and ammonium nitrate (mean numbers = 260–509). No stromata were produced on media in which cystine or ammonium sulphate represented the only source of nitrogen. Ascocarps formed in significantly higher ($P \leq 0.05$) percentages of stromata when $NaNO_3$ was replaced with ammonium tartrate (18%–22%), glutamic acid (16%–22%), or serine (15%–20%) (Table 2). While relatively few stromata were produced with lysine as the source of nitrogen, ascocarps formed in 9% and 14% of the stromata produced by cultures of *P. alliaceus* isolated from cotton field soils in eastern New Mexico. No ascocarps were found in stromata produced on media containing cysteine.

Fig. 1. Stromata of *Petromyces alliaceus* NRRL 31814 produced on Czapek agar with (A) $NaNO_3$ or (B) lysine as the source of nitrogen (14 days, 30 °C).



The large majority of stromata produced on the different culture media and sources of nitrogen, save for leucine, measured 1.5–2.0 mm in length. At the same time, ascocarps were recorded from stromata of all dimensions. In many instances, stromata adhering to one another in clusters might all be found to contain ascocarps, while only one or two ascocarps were found in other groups of adhering groups of stromata from the same culture plate.

Discussion

This research has identified culture media containing a variety of natural materials or sources of nitrogen that promote the formation of stromata with ascocarps in *P. alliaceus*. The largest numbers of *P. alliaeus* stromata per Petri dish were recorded for MCA, CZA, and CZA with leucine, glycine, or potassium nitrate substituted for $NaNO_3$. Stromata produced with leucine were consistently smaller in size when contrasted with the majority of stromata produced on the other media. Agnihotri (1968) showed that the dry weight of *Aspergillus niger* Van Tieghem sclerotia varied depending on the source of nitrogen used in the culture medium, and on media where maximum numbers of sclerotia were recorded, average sclerotial size was substantially reduced. Rudolph (1962) studied the effect of carbon–nitrogen balance, temperature, pH, and light on the formation of sclerotia by *P. alliaceus* NRRL 315 and five other species of *Aspergillus*. High nitrate (0.3%–0.6% $NaNO_3$) and high sucrose (10%–20%) concentrations were optimal for stromata/sclerotium development on CZA. *Aspergillus flavus* Link NRRL 3357 also produced the largest numbers of sclerotia on CZA with or without potassium nitrate or glycine substituted for $NaNO_3$ (McAlpin 2001). The numbers of sclerotia produced by *A. flavus* NRRL 3517 and *Aspergillus parasiticus* Speare NRRL 3145 on CZA were the greatest with 5% sucrose in the medium and 0.5% $NaNO_3$ as contrasted with any combination of these ingredients at lower concentrations (Hesseltine et al. 1970). A sclerotium-forming culture of *A. niger* received from K.B. Raper produced the largest numbers of sclerotia on a basal medium consisting of glucose, $NaNO_3$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, agar, and distilled water in which histidine was substituted for $NaNO_3$ (Agnihotri 1968). No sclerotia were produced by *A. flavus* on CZA with either leucine or histidine substituted for $NaNO_3$ (McAlpin 2001). No stromata were produced by *P. alliaceus* on media in which cystine or ammonium sulphate represented the only source of nitrogen. Nitrogen sources supporting the fewest *P. alliaceus* stromata per culture plate included cysteine, lysine, and ammonium nitrate.

Some ascomycetes may require exogenous vitamins, minerals, or other natural materials for ascocarp production, often not duplicated in synthetic media (Moore-Landecker 1992). MCA is a complex medium prepared from rice, wheat, and oats and supplemented with vitamins and minerals. The percentage of *P. alliaceus* stromata containing ascocarps was the greatest for MCA and for CZA with either ammonium tartrate, glutamic acid, glycine, or serine substituted for $NaNO_3$. To our knowledge, ammonium tartrate has not been associated with initiation of the sexual cycle in any fungus. No ascocarps developed in any of the stromata produced on media containing cysteine. The precise role of these nitrogen sources in stimulating ascocarp formation is not known. In studies with other ascomycetes, these same sources of nitrogen were associated with high ascocarp production in *Sordaria fimicola* (Rge) Cesati et de Notaris, glycine and glutamic acid (Hodgkiss 1969) but low ascospore or ascocarp production in *Talaromyces flavus* (Klocker) Stolk & Samson, ammonium tartrate, glutamic acid, glycine, and serine (Engelkes et al. 1997) in *Pyrenoma domesticum* (Sow.) Sacc., and glycine (Moore-Landecker 1987) in *Hypomyces solani* (Mart.) App. & Wr. f. *cucurbitae* Snyd. & Hans. (Hix and Baker 1964). *Sporormiella* spp. and *Podospora anserina* (Ces. in Rabenh.) Niessl. produced no ascocarps with glutamic acid or glycine as the source of nitrogen (Hodgkiss 1969; Asina et al. 1977), while *Venturia inaequalis* (Cke.) Wint. produced large numbers of ascocarps with glycine but no ascocarps with ammonium tartrate (Ross and Bremner 1971). *Venturia inaequalis* fails to form ascocarps on medium containing ammonium salts unless calcium carbonate is added to control the unfavorably low pH that would otherwise result in the medium (Ross and Bremner 1971; Moore-Landecker 1992). This same phenomenon was demonstrated to be responsible for ammonium suppression of sclerotium formation in *A. flavus* and *A. niger* (Agnihotri 1968; Cotty 1988).

No evidence was found that any of the culture media supported a more rapid rate of ascocarp ripening among the three isolates of *P. alliaceus* that we examined. Fennell and Warcup (1959) reported stromata containing ascocarps with asci and ascospores for cultures of *P. alliaceus* grown on CZA at 25 °C following 3 months of incubation for QM 1892 (= SA 117, WB 4181, NRRL 4181) isolated from soil, Mt. Riddock, Australia, and 10 months of incubation for QM 1885 (= C. Thom 4656, NRRL 315) isolated from a dead blister beetle (*Epicauta albida* Say., Coleoptera, Meloidae), Washington, DC. Tewari (1983, 1985) reported stromata containing young ascocarps after 4 weeks and mature ascocarps after 8 weeks for cultures of *P. alliaceus* (syn. *Petromyces albertensis* Tewari) UAMH 2976 (= ATCC 58745, NRRL 20602) grown on "Pablum" cereal agar at 22 °C. Tewari (1985) reported that when cultured on CZA, the fungus "differentiates much more slowly". This culture of *P. alliaceus* was isolated from a human ear swab and produces atypical irregularly shaped and indeterminately growing stromata.

The information should prove useful in supporting genetic investigations on the significance of recombinant diversity in ochratoxin A production (sensu Geiser et al. 1998) and in interpreting the contributions of fertile and nonfertile stromata in the fungal disease cycle that, like other species in *Aspergillus* section *Flavi*, may include insects as potential hosts (Lussenhop and Wicklow 1990; Wicklow 1993; Wicklow et al. 1993). No one has reported the natural occurrence of *P. alliaceus* stromata. However, the discovery of substantial ochratoxin A contamination with bright greenish yellow fluorescence (BGYF) in several California figs (*F. carica*) infested with *P. alliaceus* (Doster et al. 1996; Doster and Michailides 1998) will invite further research on the *P. alliaceus* disease cycle (Bayman et al. 2002). This will require an examination of the significance of stromata in fungal survival (Laakso et al. 1994) and ascospores as infective inoculum. Rudolph (1962) theorized that sclerotia in *Aspergillus* might constitute sterile stromata, and in the past, some aspergilli that had stromata containing cleistothecia and asci lost their sexual mechanism and were left with sterile stromata, which have been termed "sclerotia". In the present study, we have shown that only a portion of the stromata produced in vitro eventually ripened to form ascocarps. In nature, stromata that ripen to produce ascocarps and ascospores would become softened and more likely to decompose or disintegrate. At the same time, infertile stromata should remain hardened,

functioning as sclerotia and contributing to long-term fungal survival. *Pyronema domesticum* forms both apothecia and sclerotia in vitro, and whether the culture will form apothecia or sclerotia is determined early and is nonreversible (Moore-Landecker 1987). Such a reproductive strategy would spread the risk of fungal survival. Alternatively, some stromata may incorporate mechanisms of delayed maturation, only ripening following exposure to environmental cues.

Geiser et al. (1998) presented evidence that among isolates of *A. flavus* belonging to the same reproductively isolated clade, the genealogies of unlinked genes were significantly different from one another, which is consistent with a history of genetic recombination. The authors suggested that, under some conditions in nature, the sclerotia of *A. flavus* may reveal a teleomorphic state as with stromata in *P. alliaceus* (Warcup and Fennell 1959; Malloch and Cain 1972). Culture media that promote ascocarp production in *P. alliaceus* have the potential to induce ascocarp production in *A. flavus* and other "sclerotium"-producing species of *Aspergillus*.

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